CHAPTER – 4
GENERAL SUMMARY

The general outcome of the present work is briefly outlined below:

Although classical genetics has direct and major roles in crop improvement programs through plant breeding, a number of aspects related to the gene expression such as effect of a particular gene in a background of other genes, their nature and regulation of gene expression remain unanswered. Marker Assisted Selection (MAS) and map based cloning can provide us with tools to address these aspects. Classical breeding has not able to provide chickpea genotypes completely resistant against *Fusarium* wilt which is the major production constraint. The problem is further compounded because *Fusarium* is known to have eight physiological races.

Thus cloning of wilt resistance genes is desirable to obtain commercial variety giving resistance to multiple races of *Fusarium oxysporium* f. sp. *ciceris* and giving high yield through disease prevention. Through MAS multiple desirable traits could be introgressed into a single line and for MAS closely linked molecular markers to the trait of interest is essential. The present study was aimed at tagging STMS markers to loci giving resistance against *Fusarium* wilt race-1 infection. As an outcome of the study a STMS marker TA37 was found to be tagged with the phenotypic marker for *Fusarium* wilt race one resistance (*foc*-1) at a genetic distance of 0.2 cM.
A high density map is the first step towards genomics applications for crop improvement. Hence the study was aimed at finding as many closely linked markers to the phenotypic marker as possible. Besides TA37 two other markers TA200 and TR 2 were found flanking the foc-1 loci. TA37 and TA200 together could be helpful in MAS in breeding chickpea variety resistant against *Fusarium* wilt.

Closely linked molecular marker in a linkage map will also help in physical mapping. A physical map will provide an invaluable and readily accessible system for many detailed genetic studies and isolation of genes of economic and biological importance. MAS have made breeding more efficient, error free, less labourious and particularly less time consuming. This is so because unlike in conventional breeding, where selection is based on screening of field grown crop, in MAS. The same can be supplemented by laboratory based molecular analysis by possibly growing a crop in field, provided a reliable marker is available. STMS markers are advantageous compared to other markers as because they are co-dominant, locus specific and detection method does not involve radioactive materials, besides giving whole genome coverage.

As *Fusarium* wilt resistance loci were reported to be recessive in nature a co-dominant marker system like STMS would provide molecular tags to clone the recessive allele. Moreover, the resistance against the pathogen is race-specific; changes in the Avr gene in the pathogen may result in the loss of resistance in the host plant. Introgression of different race-specific resistance into one variety may be the solution for prolonged resistance of a variety against the pathogen. For introgression of number of resistance loci by gene pyramiding into one single
variety MAS is essential. Thus a co-dominant marker like STMS could be very useful in obtaining commercial chickpea variety with multiple disease resistance through introgression of recessive alleles.

Seed protein profiles are highly specific stable, unaffected by environmental factors, cultivation practice, age of the plant and hence reflect the genetic set up of the plant. Works in this area gained momentum after International Seed Testing Association endorsed it as a reliable technique to assess and characterise cultivars of crop plants. Moreover, there are instances of specific seed protein associated with specific traits like Mungbean Yellow Mosaic Virus (MYMV), Cercosperma Leaf Spot (CLP) of Mungbean. Hence, it is possible to generate stable protein marker for disease resistance which can be experimentally determined. In the present study diverse types of genotypes were taken viz. Resistant, moderately resistant, susceptible, and highly susceptible. Out of the total 26 samples, 13 were parental genotypes and rest were RILs. The protein profile showed lot of diversity although few exhibited identical profile. Therefore, as a whole, the protein profile diversity is reflective of the genetic diversity of the genotypes tested. The findings are in the line with similar report for inter-specific diversity in seed legumes by earlier workers. However, no seed protein was found to be associated with *Fusarium* wilt resistance or susceptibility.

Disease resistances in plants are complex and are oligogenic in nature. They do not manifest simple Mendelian inheritance and are subjected to multiple Gene x Environment interactions. Classical quantitative genetics through statistical analysis can infer function of single locus which inherits in Mendelian order. But it is not possible to deduce their interactions with each other. Cloning
and characterization of R-genes in related species can also be helpful in deducing resistance in another crop plant. *In silico* analysis was carried out to sort out putative R-gene for *Fusarium* wilt resistance from publicly available EST database of chickpea. The putative R-gene was further analysed for its protein product in terms of amino acid sequences and 3D structure to understand how it work or signal the process of disease resistance. R-FOM-2 gene is known to be the gene that confers resistance to *Fusarium* wilt in melon. Using R-FOM-2 as a reference sequence, databases were searched for putative chickpea sequence homologous to R-gene for resistance against *Fusarium* wilt in melon. The putative sequence could be an important tool for designing gene specific primer sequences for PCR based cloning of R-genes in chickpea as well as marker.